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## Structure elucidation of seven steroids from *Sinularia conferta*

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### Abstract

Seven steroids were isolated from the methanol extract of the soft corals *Sinularia conferta*. These steroids were elucidated as 7 $\alpha$ -methoxyergosta-5,24(28)-diene-3 $\beta$ -ol (**1**), ergosta-5-ene-3 $\beta$ ,7 $\alpha$ -diol (**2**), 3 $\beta$ ,7 $\alpha$ -dihydroxyergosta-5,24(28)-diene (**3**), 3 $\beta$ -hydroxyergosta-5,24(28)-diene-7-one (**4**), ergosta-24(28)-ene-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol-6-acetate (**5**), ergosta-24(28)-ene-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol (**6**), and ergosta-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol (**7**) by 1D and 2D-NMR experiments and comparison with reported data.

**Keywords.** *Sinularia conferta*, Alcyoniidae, soft coral, steroid.

### 1. INTRODUCTION

The phylum Cnidaria are traditionally divided into three classes: Anthozoa, Hydrozoa, and Scyphozoa. The best-known representatives of the class Anthozoa are the gorgonians and soft corals. Soft corals attracted considerable attention because of the wide range of their bioactive secondary metabolites. Among these marine invertebrates, genus *Sinularia* is one of the most widely distributed soft corals and constitutes a dominant portion of the biomass in the tropical reef environment [1, 2]. Prior investigations demonstrated that *Sinularia* species are a rich source of steroids and terpenoids [2-5].

As a part of our recent investigations on chemical constituents and biological activities of Vietnamese *Sinularia* soft corals, we have recently reported twelve steroids from the *Sinularia conferta* species and their *in vitro* cytotoxicity [6]. The current paper deals with detailed structure elucidation of seven steroids from this species.

### 2. EXPERIMENTAL

#### 2.1. General experimental procedures

The <sup>1</sup>H-NMR (500 MHz) and <sup>13</sup>C-NMR (125 MHz) spectra were recorded on a Bruker AM500 and AVANCE III HD 500 FT-NMR spectrometers with

TMS used as an internal standard. Medium pressure liquid chromatography (MPLC) was carried out on a Biotage - Isolera One system (SE-751 03 Uppsala, Sweden). Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck, Darmstadt, Germany), YMC\*GEL (ODS-A, 12 nm S-150 mm, YMC Co., Ltd., Japan), Sephadex LH-20 (Sigma-Aldrich, USA), and Diaion HP-20 (Supelco, USA) resins. Thin layer chromatography (TLC) used pre-coated silica gel 60 F<sub>254</sub> (1.05554.0001, Merck) and RP-18 F<sub>254S</sub> plates (1.15685.0001, Merck). Compounds were visualized by spraying with aqueous 10 % H<sub>2</sub>SO<sub>4</sub> and heating for 3-5 minutes.

#### 2.2. Marine materials

The samples of *S. conferta* (Dana, 1846) were collected near the Con Co island, Quangtri, Vietnam, in May 2015, and identified by Prof. Do Cong Thung, Institute of Marine Environment and Resources, VAST. A voucher specimen (SCO-052015) was deposited at the Institute of Marine Environment and Resources and Institute of Marine Biochemistry, VAST, Vietnam.

#### 2.3. Isolation

Dried bodies of the soft coral *S. conferta* (2.5 kg) were extracted three times with methanol (5 L each)

under ultrasonic condition. The resulting solutions were filtered, combined, and concentrated under reduced pressure to give the methanol residue (SCO.M, 150.0 g), which was suspended in water

and extracted in turn with *n*-hexane and CH<sub>2</sub>Cl<sub>2</sub> resulting in extracts of *n*-hexane (SCO.H, 100.0 g), CH<sub>2</sub>Cl<sub>2</sub> (SCO.D, 6.0 g), and water layer.

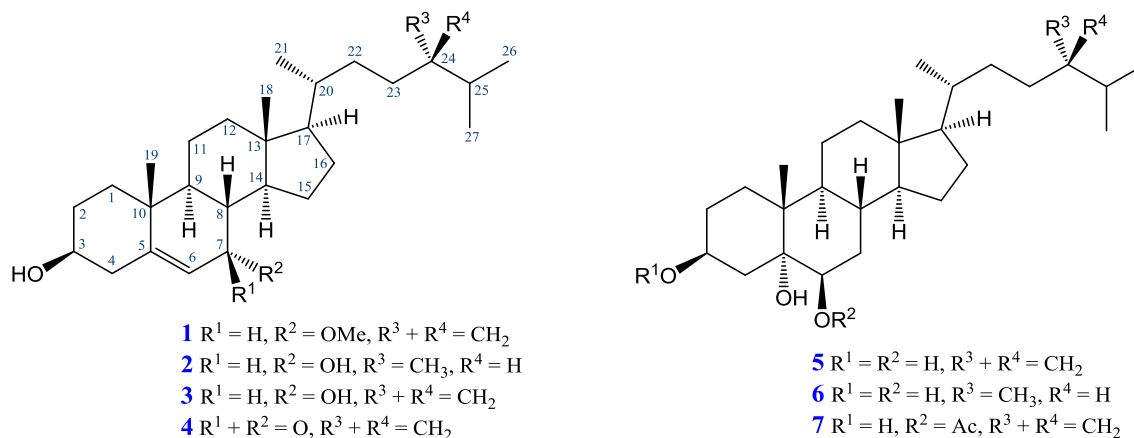


Figure 1: Chemical structures of compounds **1-7**

Table 1: <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>) data of **1** and **2**

C	<sup>a</sup> δ <sub>C</sub>	<b>1</b>		<sup>b</sup> δ <sub>C</sub>	<b>2</b>		
		δ <sub>C</sub>	δ <sub>H</sub> mult. (J = Hz)		δ <sub>C</sub>	δ <sub>H</sub> mult. (J = Hz)	
1	37.1	36.77	1.16 m/1.83 m	37.6	37.04	1.12 m/1.86 m	
2	31.4	31.49	1.52 m/1.85 m	32.4	30.44	0.95 m/1.38 m	
3	71.2	71.46	3.62 m	71.0	71.38	3.59 m	
4	42.1	42.33	2.30 m/2.33 m	43.4	42.04	2.28 m/2.33 m	
5	146.2	146.15	-	144.9	146.26	-	
6	121.5	120.78	5.73 dd (1.5, 4.0)	125.5	123.90	5.61 dd (1.5, 5.0)	
7	73.7	73.92	3.29 br s	64.8	65.38	3.85 br s	
8	37.2	37.24	1.49 m	38.4	37.56	1.48 m	
9	42.4	42.76	1.32 m	42.7	42.30	1.23 m	
10	37.1	37.47	-	37.7	37.42	-	
11	20.6	20.84	1.45 m/1.50 m	21.2	20.73	1.50 m/1.54 m	
12	38.9	39.09	1.18 m/2.17 m	39.8	39.10	1.17 m/2.00 m	
13	42.0	42.16	-	42.3	42.16	-	
14	48.8	49.09	1.50 m	50.2	49.44	1.44 m	
15	24.1	24.27	0.98 m/1.63 m	24.7	24.32	1.05 m/1.73 m	
16	28.1	28.23	1.28 m/1.90 m	28.7	28.24	1.31 m/1.90 m	
17	55.5	55.67	1.21 m	56.0	55.70	1.02 m	
18	11.4	11.49	0.67 s	12.0	11.65	0.68 s	
19	18.1	18.27	0.99 s	18.5	18.26	1.00 s	
20	35.6	35.77	1.43 m	36.5	36.16	1.38 m	
21	18.6	18.76	0.95 d (6.5)	19.2	18.93	0.93 d (6.5)	
22	34.7	34.72	1.16 m/1.55 m	34.0	33.73	0.98 m/1.40 m	
23	30.9	30.91	1.88 m/2.10 m	30.8	31.41	1.52 m/1.86 m	
24	156.7	156.97	-	39.3	39.20	1.21 m	
25	33.7	33.86	2.23 m	31.7	31.52	1.58 m	
26	21.8	21.89	1.02 d (6.5)	17.7	17.66	0.79 d (6.5)	
27	21.9	22.03	1.03 d (6.5)	20.7	20.50	0.86 d (6.5)	
28	105.8	105.92	4.44 s/4.71 s	15.6	15.49	0.78 d (6.5)	
OMe	56.6	56.78	3.35 s				

<sup>a</sup>δ<sub>C</sub> of 7α-methoxyergosta-5,24(28)-diene-3β-ol in CDCl<sub>3</sub> [7], <sup>b</sup>δ of ergost-5-en-3β,7α-diol in pyridine-*d*<sub>5</sub> [8].

Table 2:  $^1\text{H}$ -NMR (500 MHz,  $\text{CD}_3\text{OD}$ ) and  $^{13}\text{C}$ -NMR (125 MHz,  $\text{CD}_3\text{OD}$ ) data of **5** and **6**

C	$^a\delta_{\text{C}}$	<b>5</b>		$^b\delta_{\text{C}}$	<b>6</b>	
		$\delta_{\text{C}}$	$\delta_{\text{H}}$ mult. ( $J = \text{Hz}$ )		$\delta_{\text{C}}$	$\delta_{\text{H}}$ mult. ( $J = \text{Hz}$ )
1	33.3	33.49	1.36 m/1.61 m	33.3	33.48	1.35 m/1.60 m
2	32.5	31.69	1.52 m/1.78 m	32.5	31.62	1.00 m/1.50 m
3	67.4	68.33	4.03 m	67.4	68.33	4.02 m
4	42.9	41.44	1.20 m/2.08 m	42.9	41.44	1.54 m/2.08 m
5	75.9	76.81	-	75.9	76.82	-
6	76.3	76.53	3.47 br s	76.3	76.53	3.47 br s
7	35.7	35.28	1.55 m/1.72 m	35.7	35.29	1.52 m/1.71 m
8	31.4	31.62	1.74 m	31.2	31.69	1.71 m
9	45.9	46.57	1.39 m	45.9	46.58	1.39 m
10	39.1	39.31	-	39.2	39.31	-
11	21.8	22.31	1.39 m	21.8	22.30	1.40 m
12	40.7	41.50	1.56 m/2.03 m	40.7	41.49	1.19 m/2.03 m
13	43.1	43.96	-	43.1	43.91	-
14	56.5	57.54	1.16 m	56.5	57.55	1.13 m
15	24.6	25.22	1.13 m/1.63 m	24.6	25.22	1.12 m/1.60 m
16	28.6	29.33	1.30 m/1.88 m	28.6	29.31	1.31 m/1.87 m
17	56.6	57.45	1.13 m	56.6	57.46	1.13 m
18	12.4	12.62	0.74 s	12.4	12.50	0.73 s
19	17.2	17.31	1.18 s	17.2	17.20	1.17 s
20	36.1	36.99	1.45 m	36.6	37.53	1.40 m
21	18.9	19.21	0.99 d (6.5)	19.1	19.20	0.96 d (6.5)
22	35.1	36.02	1.17 m/1.58 m	34.0	34.93	1.00 m/1.46 m
23	31.2	32.11	1.93 m/2.14 m	31.0	30.80	1.31 m
24	156.7	157.78	-	39.4	40.44	1.24 m
25	34.1	34.91	2.25 m	31.7	32.71	1.60 m
26	22.1	22.30	1.04 d (6.5)	17.8	18.00	0.83 d (6.5)
27	22.0	22.47	1.05 d (6.5)	20.7	20.86	0.90 d (6.5)
28	106.6	106.88	4.67 br s/4.74 br s	15.7	16.00	0.83 d (6.5)

$^a\delta_{\text{C}}$  of ergosta-24(28)-ene-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol in pyridine- $d_5$  [8];  $^b\delta_{\text{C}}$  of ergosta-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol in pyridine- $d_5$  [9].

Extract SCO.H (100.0 g) was fractionated on silica gel MPLC using the mobile phase of *n*-hexane-acetone (gradient 50:1→1:1, v/v) to yield eight fractions, SCO.H1–SCO.H8. Fraction SCO.H5 (10 g) was further subjected to RP-18 MPLC using the mobile phase of MeOH–H<sub>2</sub>O (gradient 2:1→10:1, v/v) to give seventeen subfractions, SCO.H5A1–SCO.H5A17. Subfraction SCO.H5A14 (800 mg) was fractionated into ten smaller fractions, SCO.H5A14A–SCO.H5A14K, by silica gel CC using CH<sub>2</sub>Cl<sub>2</sub>–acetone (10:1, v/v) as eluent. Fraction SCO.H5A14C (80 mg) was purified by YMC CC eluting with acetone–H<sub>2</sub>O (5:1, v/v), followed by silica gel CC with *n*-hexane-ethyl acetate (4.5:1, v/v) to afford compound **1** (3.0 mg). Fraction SCO.H5A14F (100 mg) was further fractionated by silica gel CC with *n*-hexane-acetone (2:1, v/v), followed by YMC CC eluting with acetone–H<sub>2</sub>O (4:1, v/v), to furnish compound **4** (5.0 mg). Compounds **2** (1.0 mg) and **3** (2.0 mg) were purified

from fraction SCO.H5A14H (60 mg) after subjecting it on YMC CC eluting with acetone–H<sub>2</sub>O (5:1, v/v). Fraction SCO.H5A14K (50 mg) was purified by silica gel CC eluting with *n*-hexane–acetone (5:1, v/v) to give compound **7** (9.5 mg). Subfraction SCO.H5A12 (700 mg) was fractionated into five smaller fractions, SCO.H5A12A–SCO.H5A12E, by silica gel CC with CH<sub>2</sub>Cl<sub>2</sub>–acetone (10:1, v/v). Fraction SCO.H5A12K (34 mg) was purified by silica gel CC eluting with *n*-hexane-acetone (3:1, v/v), followed by YMC CC with methanol–H<sub>2</sub>O (10:1, v/v), to give compounds **5** (2.4 mg) and **6** (1.0 mg).

**7 $\alpha$ -Methoxyergosta-5,24(28)-diene-3 $\beta$ -ol (1):** White powder;  $^1\text{H}$ -NMR (500 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$ -NMR (125 MHz,  $\text{CDCl}_3$ ) see table 1; ESI-MS  $m/z$  429  $[\text{M}+\text{H}]^+$  ( $\text{C}_{29}\text{H}_{48}\text{O}_2$ ,  $M = 428$ ).

**Ergosta-5-ene-3 $\beta$ ,7 $\alpha$ -diol (2):** White powder;  $^1\text{H}$ -NMR (500 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$ -NMR (125

MHz,  $\text{CDCl}_3$ ) see table 1; ESI-MS  $m/z$  399  $[\text{M}-\text{H}_2\text{O}+\text{H}]^+$  ( $\text{C}_{28}\text{H}_{48}\text{O}_2$ ,  $M = 416$ ).

**Ergosta-24(28)-ene-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol (5):** White powder;  $^1\text{H}$ -NMR (500 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$ -NMR (125 MHz,  $\text{CDCl}_3$ ) see table 2; ESI-MS  $m/z$  433  $[\text{M}+\text{H}]^+$  ( $\text{C}_{28}\text{H}_{48}\text{O}_3$ ,  $M = 432$ ).

**Ergosta-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol (6):** White powder;  $^1\text{H}$ -NMR (500 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$ -NMR (125 MHz,  $\text{CDCl}_3$ ) see table 2; ESI-MS  $m/z$  435  $[\text{M}+\text{H}]^+$  ( $\text{C}_{28}\text{H}_{50}\text{O}_3$ ,  $M = 434$ ).

### 3. RESULTS AND DISCUSSION

Compound **1** was isolated as a white powder. Its NMR features are indicative for an ergosterol derivative, one main constituent of soft corals [5]. The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra revealed typical signals of two oxymethines [ $\delta_{\text{C}}$  71.46 (C-3) and 73.92 (C-7)/ $\delta_{\text{H}}$  3.62 (1H, m, H-3) and 3.29 (1H, br s, H-7)], one trisubstituted double bond [ $\delta_{\text{C}}$  146.15 (C, C-4) and 120.78 (CH, C-5)/ $\delta_{\text{H}}$  5.73 (1H, dd,  $J = 1.0$ , 4.0 Hz, H-6)], one terminal disubstituted double bond [ $\delta_{\text{C}}$  156.97 (C, C-24) and 105.92 ( $\text{CH}_2$ , C-28)/ $\delta_{\text{H}}$  4.44 and 4.71, each 1H, s, H-28], two *tert*-methyls [ $\delta_{\text{C}}$  11.49 (C-18) and 18.27 (C-19)/ $\delta_{\text{H}}$  0.67 (H-18) and 0.99 (H-19), each 3H, s], three *sec*-methyls [ $\delta_{\text{C}}$  18.67 (C-21), 21.89 (C-26), and 22.03 (C-27)/ $\delta_{\text{H}}$  0.95 (H-21), 1.02 (H-26), and 1.03 (H-27), each 3H, d,  $J = 6.5$  Hz], and one methoxy group [ $\delta_{\text{C}}$  56.78/ $\delta_{\text{H}}$  3.35 (3H, s)].

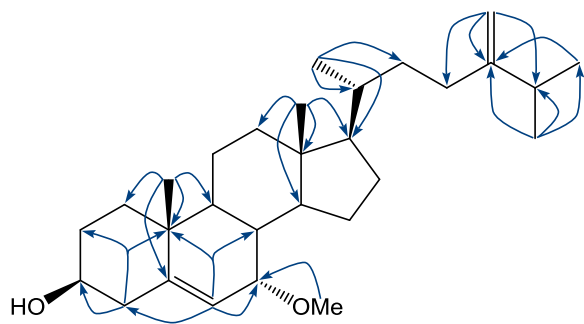


Figure 2: Key HMBC (H  $\rightarrow$  C) correlations of **1**

The HMBC cross-peaks of H-19 with C-5 and H-6 with C-8 and C-10 confirmed the position of the trisubstituted double bond at C-5/C-6. The disubstituted double bond was identified at C-24/C-28 by HMBC cross-peaks of H-28 with C-23 and C-25 and H-26/H-27 with C-24 and C-25. The HMBC correlation of the methoxy proton with C-7 confirming attachment of the methoxy group at this carbon. Comparison of the  $^{13}\text{C}$ -NMR data of **1** with those reported (table 1) and detailed analysis of the other HMBC cross-peaks (Fig. 2) led to the

identification of **1** as 7 $\alpha$ -methoxyergosta-5,24(28)-diene-3 $\beta$ -ol [7, 10].

The NMR data of **2** were similar to those of **1**, except for absence of the methoxy group and presence of a methine and a *sec*-methyl in **2** instead of a terminal double bond in **1**. Comparison of the  $^{13}\text{C}$ -NMR data of **2** with those reported (table 1) and detailed analysis of the HSQC and HMBC correlations led to the identification of **2** as ergost-5-en-3 $\beta$ ,7 $\alpha$ -diol [8].

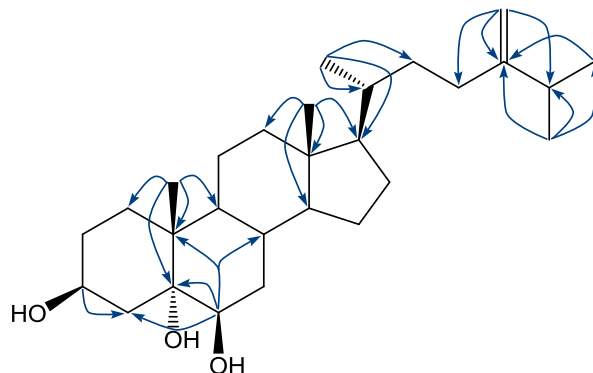


Figure 3: Key HMBC (H  $\rightarrow$  C) correlations of **5**

The NMR features of **5** are also indicative for an ergosterol derivative. The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra revealed typical signals of two oxymethines [ $\delta_{\text{C}}$  68.33 (C-3) and 76.53 (C-6)/ $\delta_{\text{H}}$  4.03 (1H, m, H-3) and 3.47 (1H, br s, H-6)], one oxygenated quaternary carbon [ $\delta_{\text{C}}$  76.81 (C-5)], one terminal disubstituted double bond [ $\delta_{\text{C}}$  157.78 (C, C-24) and 106.88 ( $\text{CH}_2$ , C-28)/ $\delta_{\text{H}}$  4.67 and 4.74, each 1H, s, H-28], two *tert*-methyls [ $\delta_{\text{C}}$  12.62 (C-18) and 17.31 (C-19)/ $\delta_{\text{H}}$  0.74 (H-18) and 1.18 (H-19), each 3H, s] and three *sec*-methyls [ $\delta_{\text{C}}$  19.21 (C-21), 22.30 (C-26), and 22.47 (C-27)/ $\delta_{\text{H}}$  0.99 (H-21), 1.04 (H-26), and 1.05 (H-27), each 3H, d,  $J = 6.5$  Hz]. Comparison of the  $^{13}\text{C}$ -NMR data of **5** with those reported (Table 2) and detailed analysis of the HSQC and HMBC correlations (Fig. 3) led to identification of **5** as ergosta-24(28)-ene-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol [8].

Compound **6** was also isolated as a white powder. Its NMR data were similar to those of **5** (table 2), except for the presence of a methine and a *sec*-methyl in **6** instead of a terminal double bond in **5**. Comparison of the  $^{13}\text{C}$ -NMR data of **6** with those reported (table 2) and detailed analysis of the HSQC and HMBC correlations led to the identification of **6** as ergosta-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol [9].

The other compounds were elucidated as 3 $\beta$ ,7 $\alpha$ -dihydroxyergosta-5,24(28)-diene (**3**) [11, 12], 3 $\beta$ -hydroxyergosta-5,24(28)-diene-7-one (**4**) [13, 14], and ergosta-24(28)-ene-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol-6-acetate (**7**) [12, 15] by comparison of their  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR

values with those reported and detailed analysis of HSQC and HMBC correlations.

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